

# Reproducibility of Glucose Tolerance in 101 Nondiabetic Women

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WITH THE increasing use of the oral glucose tolerance test for diabetes screening purposes, due in part to automated techniques, the reproducibility of present oral glucose tolerance test screening methods has been questioned. The present method of testing each subject once, and then giving a glucose tolerance test to those whose screening level appears abnormal, often yields a normal GTT on the retest, even when the persons tested have characteristics associated with diabetes, such as obesity or a family history of the disease.

This paper concerns itself primarily with defining oral glucose tolerance test reproducibility by examining a select population of nondiabetic women. The study, conducted on female prisoners between July and December 1964, is a followup of a previous study of male prisoners (1). Both studies were concerned with reproducibility of test results, and so far as possible, were based upon the same experimental design.

## Description of Study

The study population was composed of a group of female prisoners who volunteered for the project. Subjects were not accepted for the project if they were expected to leave the prison within 6 months, were known to have diabetes,

or if they had other chronic conditions which might affect glucose tolerance.

The initial group was composed of 115 women. Information was collected on age, race, height, weight, activity level, smoking status, family history of diabetes, number of pregnancies, miscarriages, and whether the women had had large babies (9 pounds or more). Before each test, individual participants reported the time of their last food intake. Illness, medication, and changes in eating pattern, or changes in physical activity were recorded for the previous 2-week period. The participants were weighed before each test. Although no prescribed diet was given to the volunteers to follow before the day of the test, a review of the institution's menus by a nutritionist showed that the women were receiving carbohydrates well above the daily average of 250 to 300 gm. generally recommended for the 3-day period before a glucose tolerance test. Since the volunteers were institutionalized, there was greater assurance of proper diet than for the general population.

Between five and 10 women were tested each day. Each woman was scheduled to receive four tests, at intervals of approximately 1 month. Testing began each morning at 8 a.m. and fasting blood samples were drawn using Becton-Dickinson vacutainers, No. 3204, 7 cc. These vacutainers contained 50 mg. of sodium fluoride as a preservative and 5 mg. of ethylenediamine tetracetic acid as an anticoagulant. Next, a 100

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gm., 50 percent glucose solution was administered. Venous blood samples were drawn at fasting, 1/2, 1, 2, and 3 hours after the glucose loading. During the testing period, all volunteers remained in the testing area.

Blood samples were processed on the Technicon AutoAnalyzer (Hoffman ferricyanide macromethod) on whole blood, according to the procedures recommended by the manufacturer at the time of the study. Most of the determinations were made the same day the blood samples were drawn, and the 1-hour specimen was separated into two sample cups and two readings were obtained. The standard deviation of the duplicates ( $S_d$ ), as measured by the differences between the first and second aliquot ( $d$ ), was 2 mg. per 100 ml. Specifically, this was defined as the square root of the sum of the differences squared, divided by twice the number of pairs ( $\sqrt{\frac{\sum d^2}{2N}}$ ).

Because it was not feasible to use whole blood controls in this study, a control serum pool was prepared with an approximate value of 150 mg. per 100 ml. Standards and one control serum were processed before each set of five to 10 unknowns. Analysis of 246 control serum readings for the four tests revealed a standard deviation of 4.22 mg. per 100 ml.

## Results

*Study variables.* Four complete tests were administered to each of the 101 women. Of the 115 women originally participating, 14 were dropped. Seven women were paroled, one was released, and six started the series after the first test had already been given.

The age range of the participating women was from 17 to 59 years; age and other charac-

**Table 1. Characteristics of the 101 women in the study**

Characteristics	Number
Age in years:	
Under 25.....	21
25-29.....	24
30-34.....	16
35-39.....	16
40-44.....	13
45 or older.....	11
Weight status <sup>1</sup> :	
Underweight.....	4
Normal.....	41
Overweight.....	56
Activity level rating <sup>2</sup> :	
Moderate.....	38
Active.....	44
Very active.....	19
Race:	
White.....	44
Nonwhite.....	57
Other factors:	
Nonsmokers.....	31
Family history of diabetes.....	18
History of large babies (9 pounds or more).....	25
History of miscarriages.....	28

<sup>1</sup> Reference 2.

<sup>2</sup> Subjective evaluation by nurse acquainted with all participants.

teristics are given in table 1. The women were relatively young, physically active, and definitely overweight.

*Population distribution of glucose values.* Table 2 shows the means and standard deviations for the five readings taken during the four tests. Although 1/2-hour means were greater than first hour means, the 1/2-hour standard deviations for the four tests were smaller. Also, the results for all the hourly means were consistently higher than those of the male prisoners previously studied. These differences should be interpreted with caution since they may merely arise as a result of the slight modifications made

**Table 2. Group means and standard deviations (mg. per 100 ml.) for 101 women completing the four glucose tests<sup>1</sup> by time after glucose load**

Test	Fasting		1/2 hour		1st hour		2d hour		3d hour	
	Means	S.D.	Means	S.D.	Means	S.D.	Means	S.D.	Means	S.D.
1.....	82.7	9.2	120.4	22.9	117.6	31.5	103.0	21.9	89.6	19.6
2.....	81.1	8.8	118.1	22.9	111.3	32.5	97.3	25.9	85.5	22.5
3.....	81.5	8.4	113.9	24.2	112.7	29.8	93.5	22.3	81.5	17.1
4.....	80.3	8.0	115.5	18.8	109.5	28.0	94.2	19.3	81.5	16.5

<sup>1</sup> Whole blood processed on AutoAnalyzer.

in the study protocol or they may reflect laboratory differences.

*Variation of results in individual persons.* The reproducibility of each woman's test was obtained by measuring the standard deviation around her mean score for the four tests. The range of averages for individual persons, based on four tests, by hour, after glucose load was as follows:

Tests	Range of individual means (mg. per 100 ml.)	Median values (mg. per 100 ml.)
Fasting.....	68.5-125.0	80.5
½-hour.....	81.0-180.5	117.5
1-hour.....	63.5-192.0	109.3
2-hour.....	64.8-170.5	93.5
3-hour.....	47.8-151.0	83.3

The range of the standard deviations for individual persons based on four tests, by hour, after glucose load was as follows:

Tests	Range of individual standard deviations (mg. per 100 ml.)	Median values (mg. per 100 ml.)
Fasting.....	0.5-13.8	3.8
½-hour.....	1.7-28.9	12.5
1-hour.....	3.9-55.2	17.3
2-hour.....	.8-62.6	11.3
3-hour.....	1.7-57.1	10.6

A lack of homogeneity is apparent since a wide range of variability is shown. For example, almost 20 percent of the population varied beyond their average plus or minus 34 mg. per 100 ml. (plus or minus two times the median individual standard deviation) at 1 hour after glucose.

*Factors associated with individual variability.* In attempting to identify factors associated with individual variability, a procedure was used to define women whose variability placed them in the upper and lower half of the total population. In defining a woman's overall variability, equal weight was given to the relative position, that is, rank, at fasting and at 1, 2, and 3 hours after receiving glucose. One-half hour readings were excluded to make results comparable with the study of male prisoners. Women in the upper and lower half of the population were then compared for these eight variables:

1. Age—less than 30, 30-39, and 40 or more years.

2. Race.

3. Weight—normal (within 10 percent of ideal weight), overweight, or underweight. Weight status was obtained by comparing heights and weights with standard tables (2) based on life expectancy rather than average weights by height and sex.

4. Level of activity—moderately active, active, or very active.

5. Whether or not there was diabetes in the family.

6. Number of pregnancies.

7. Number of miscarriages.

8. Having had babies weighing 9 pounds or more.

Analysis revealed no apparent relationship between variability (rank in population) and these variables. When the population was further divided into quartiles, analysis similarly showed no relationship between top and bottom quartile ratings and these variables.

The only relationship of any significance was between mean blood glucose values and individual variability as shown in table 3.

This relationship is confirmed by a rank correlation of 0.28 between the ranked means and ranked standard deviations (variability). This correlation was approximately the same for all hours—fasting, 1-, 2-, and 3-hour tests.

*Abnormal glucose tolerance test results.* When various interpretative criteria were applied to the glucose test results, 18 women indicated abnormality. Table 4 gives the reproducibility of the various criteria. Women positive to one or more of the indicated criteria are listed by case number. The table shows tests positive

Table 3. Number of women in various ranks of mean blood glucose levels<sup>1</sup>

Rank in—	High variability (25 percent)	Low variability (25 percent)	Total
Upper 25 percent.....	7	5	12
Middle 50 percent.....	14	9	23
Lower 25 percent.....	4	11	15
Total.....	25	25	50

<sup>1</sup> The blood glucose ranks were determined in the same manner as were standard deviation ranks for the 101 women.

to the standard recommended to the Public Health Service (3). For this standard, the critical whole blood levels are 110 mg. per 100 ml. in the fasting state, 170 at 1 hour, 120 at 2 hours, and 110 at 3 hours after ingestion of glucose.

Eighteen women had elevations for two or more readings on at least one test. Of these 18, only two showed consistent elevations for the recommended levels. Six women were diagnosable according to Public Health Service consultants, since they had at least three hourly readings above the critical levels or their fasting and third hour readings were elevated for a given test. Only two, however, of these six were diagnosable on more than one of their four tests. Results positive to a modified Fajans and Conn (4) criteria are also listed in table 4. This modification is necessary since the 1½-hour blood sample was not drawn in this study.

Critical levels used were 160 mg. per 100 ml. at 1 hour, and 120 at 2 hours after receiving glucose. Thirteen women had elevated readings on one or more tests by this criteria. Two had positive results on all four tests. The American Diabetes Association recommends diagnostic criteria of 160 mg. per 100 ml. at 1 hour and 140 mg. per 100 ml. at 2 hours after receiving glucose (5). Eight women had positive test results according to these criteria.

### Discussion

The data presented document the variability of oral glucose tolerance test results on a group of nondiabetic women. Although the variation of test results for the individual person was small at fasting, the lack of reproducibility for the individual person after a glucose challenge was evident. Most readings varied considerably beyond the technical variation as measured by the variance of the control serum and the precision of the first hour duplicates. In some persons, the glucose levels ranged from normal to abnormal for the four tests when compared with standard criteria presently used to interpret the test.

These observations strongly support conclusions arrived at in a previous study when male prisoners were given oral glucose tolerance tests. Reproducibility, or lack of it, was evident on an individual basis. In both studies, the only factor associated with any degree of significance

**Table 4. Tests positive to various criteria for diagnosing diabetes**

Case number	Criteria of Public Health Service consultants <sup>1</sup>		Modified Fajans and Conn criteria <sup>2</sup>	American Diabetes Association criteria <sup>3</sup>
	Elevated at two or more readings	Diagnosable		
4-----	2	0	2	2
15-----	2	0	1	0
16-----	4	4	4	3
18-----	1	0	1	0
24-----	2	0	2	0
29-----	2	0	0	0
39-----	4	2	3	3
46-----	1	0	1	0
55-----	2	1	4	3
69-----	1	1	1	1
75-----	2	1	2	2
80-----	1	0	0	0
87-----	1	0	0	0
88-----	1	0	1	0
101-----	1	0	1	0
105-----	1	0	0	0
107-----	1	1	1	1
109-----	2	0	0	0
Total tests positive----	31	10	24	15
Total women--	18	6	13	8

<sup>1</sup> Reference 3.

<sup>2</sup> Reference 4.

<sup>3</sup> Reference 5.

with high and low variability was the mean glucose values. However, the rank correlation was small in each study. This degree of relationship cannot indicate whether a person with low glucose values will show low variability.

Thus, freedom from diabetes cannot be predicted from a single low reading. The patient should be informed that a single negative result is not conclusive and that further observations and testing are needed. At some point in the development of diabetes, glucose test results may be elevated consistently, but one should not assume this to be true in the early stages of the disease.

### Summary

Reproducibility of the 100 gm. oral glucose tolerance test was explored in a study of 101 female nondiabetic prisoners. The women participated in a series of four individual tests for a 5-month period.

Five to ten women were tested daily, and

each woman was retested at 4-week intervals. Venous blood was drawn at fasting, 1/2, 1, 2, and 3 hours after the administration of a 100 gm. glucose drink, and the blood was processed using an AutoAnalyzer. Average blood glucose levels for the total group remained stable for the period of testing. Blood glucose levels for individual persons, however, varied considerably. On single tests, some women had borderline or diagnostic test readings, but only one woman was consistently abnormal for all tests.

Analysis of a person's variability with such factors as age, race, weight, number of pregnancies, miscarriages, and having babies of 9 pounds or more revealed no apparent relationship. The only relationship of any significance was between mean blood glucose values and individual variability. Comparisons of commonly used criteria for defining glucose abnormality showed much inconsistency in determining a person's status.

The observations strongly support conclusions of a previous glucose tolerance study of

male prisoners in which individual variability was also observed. The two studies showed that freedom from diabetes cannot be predicted from a single reading and that followup testing is needed on persons otherwise suspected of having diabetes.

#### REFERENCES

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## Rescuing College Dropouts

Bright, disturbed college dropouts will be given a second chance in an experimental program at the Hahnemann Medical College and Hospital in Philadelphia. Scientists there will evaluate the effectiveness of a work and psychotherapy program as a means of preparing talented dropouts to return to school.

The research project is supported by a \$81,679 grant for the first year from the National Institute of Mental Health. Additional support for 4 more years is planned, subject to annual review. Dr. Victor P. Satinsky, research associate professor of surgery, will direct the project.

During the first 3 years of the project, 30 students (10 each year) will work in a laboratory and clinical surgery program at Hahnemann's Cardiovascular Research Institute. During the remaining 2 years, the scientists will study the rehabilitative techniques they develop and evaluate the program.

Students who scored very high (600 or better) on the college board aptitude examina-

tion, but who left school because of an emotional crisis, will be selected for the project.

At the hospital they will conduct research experiments, help with surgical procedures, work with physicians on special projects, and perform administrative work. During this time they will receive individual or group therapy.

The researchers intend to test whether such involvement in responsible, high-status work, along with emotional support, will renew the students' self-value and stimulate their academic interests.

Contact will be maintained with each student after he leaves to compare his progress with that of other dropouts who did not participate in the work-therapy program. Other goals of the program are to encourage the students to be self-sufficient, creative, and expressive.

Besides testing the program's value, the investigators hope to learn more about the relationship between emotional problems and difficulty in college and about the factors that cause capable students to drop out of school.